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(54) Title: OPTIMIZED T-DNA TRANSFER AND VECTORS THEREFOR

(57) Abstract: The present invention relates to T-DNA vectors and methods for obtaining transgenic eukaryotes using said vectors. The transgenic eukaryotes are characterized in that they contain the T-DNA but not the illegitimately transferred vector backbone sequence. This is achieved by modifying the T-DNA borders such that they are more efficiently nicked or such that they allow elimination of illegitimately transferred vector backbone sequences by means of recombination.



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INTERNATIONAL SEARCH REPORT

International Application No
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A. CLASSIFICATION OF SUBJECT MATTER

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According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C12N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 01563 A (GODDIJN OSCAR JOHANNA MARIA ;OHL STEPHAN ANDREAS (NL); SIMONS LAMB) 14 January 1999 (1999-01-14) cited in the application page 2, line 17 -page 3, line 11 page 8, line 4 -page 9, line 10; examples 1-9	1,3,4, 20,21, 23-28
X	DE BUCK S ET AL.,: "The DNA sequences of T-DNA junctions suggest that complex T-DNA loci are formed by a recombination process resembling T-DNA integration." PLANT JOURNAL, vol. 20, November 1999 (1999-11), page 295-304 XP000907032 cited in the application the whole document	1-17, 24-28

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

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Date of the actual completion of the international search

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Inte. onal Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 99 25855 A (BASZCZYNSKI CHRISTOPHER L ;GORDON KAMM WILLIAM J (US); LYZNIK ALEX) 27 May 1999 (1999-05-27) page 4, line 1-25 page 10, line 5 -page 12, line 6; example 1	1,9,18, 19
A	----- HANSON B ET AL.,: "A simple method to enrich an Agrobacterium-transformed population for plants containing only T-DNA sequences." PLANT JOURNAL, vol. 19, September 1999 (1999-09), page 727-734 XP000907033 cited in the application the whole document	1,9,18, 19
A	----- VAN HAAREN M J J ET AL.,: "FUNCTION OF HETEROLOGOUS AND PSEUDO BORDER REPEATS IN T REGION TRANSFER VIA THE OCTOPINE VIRULENCE SYSTEM OF AGROBACTERIUM-TUMEFACIENS" PLANT MOLECULAR BIOLOGY, vol. 11, 1988, page 773-781 XP000907031 cited in the application the whole document	1,7,9, 18-20
A	----- KONONOV M E ET AL: "INTEGRATION OF T-DNA BINARY VECTOR ?BACKBONE? SEQUENCES INTO THE TOBACCO GENOME: EVIDENCE FOR MULTIPLE COMPLEX PATTERNS OF INTEGRATION" PLANT JOURNAL,GB,BLACKWELL SCIENTIFIC PUBLICATIONS, OXFORD, vol. 11, no. 5, 7 May 1997 (1997-05-07), pages 945-957, XP002052140 ISSN: 0960-7412 cited in the application the whole document	1,9, 18-20
A	----- TINLAND B ET AL: "THE AGROBACTERIUM TUMEFACIENS VIRULENCE D2 PROTEIN IS RESPONSIBLE FOR PRECISE INTEGRATION OF T-DNA INTO THE PLANT GENOME" EMBO JOURNAL,GB,OXFORD UNIVERSITY PRESS, SURREY, vol. 14, no. 14, 17 July 1995 (1995-07-17), pages 3585-3595, XP002024327 ISSN: 0261-4189 cited in the application the whole document	1,9,19, 20
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INTERNATIONAL SEARCH REPORT

International Application No

PCT/EP 00/12683

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>PERALTA E G ET AL.,: "OVERDRIVE A T-DNA TRANSMISSION ENHANCER ON THE AGROBACTERIUM-TUMEFACIENS TUMOR-INDUCING PLASMID"</p> <p>EMBO, vol. 5, 1986, page 1137-1142 XP000910809 cited in the application the whole document -----</p>	1,7,9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/EP 00/12683

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9901563 A	14-01-1999	AU 730949 B	22-03-2001
		AU 8973598 A	25-01-1999
		BR 9810368 A	29-08-2000
		CN 1261919 T	02-08-2000
		EP 1009842 A	21-06-2000
		HU 0002698 A	28-12-2000
WO 9925855 A	27-05-1999	AU 1462999 A	07-06-1999
		AU 1588499 A	07-06-1999
		AU 1590499 A	07-06-1999
		AU 1590599 A	07-06-1999
		EP 1034262 A	13-09-2000
		EP 1034286 A	13-09-2000
		EP 1032680 A	06-09-2000
		EP 1032693 A	06-09-2000
		WO 9925840 A	27-05-1999
		WO 9925854 A	27-05-1999
		WO 9925821 A	27-05-1999
		US 6187994 B	13-02-2001
		ZA 9810528 A	20-05-1999
		ZA 9810529 A	20-05-1999
		ZA 9810532 A	18-05-1999
		ZA 9810537 A	25-05-1999